Vol. 155, No. 3 December, 1978

THE
BIOLOGICAL BULLETIN

PUBLISHED BY THE MARINE BIOLOGICAL LABORATORY

BIOLUMINESCENCE AND OTHER RESPONSES SPREAD BY EPITHELIAL CONDUCTION IN THE SIPHONOPHORE *HIPPOPODIUS*

Reference: *Biol. Bull.*, **155**: 473–498. (December, 1978)

J.-M. BASSOT, A. BILBAUT,[1] G. O. MACKIE,[2] L. M. PASSANO[3] AND M. PAVANS DE CECCATTY

Laboratoire de Bioluminescence, CNRS, 91190 Gif sur Yvette, France

Conducting epithelia are known to play an important part in the behavior of several groups of animals including hydrozoan coelenterates and amphibian larvae (reviewed by Mackie, 1970; Spencer, 1974), and skin conduction has recently been reported in pelagic tunicates and ascidian tadpoles (Bone and Mackie, 1975; Mackie and Bone, 1976).

These epithelia include both simple, non-muscular sheets of cells and myoepithelia. It has been shown in a few cases that the cells are electrically coupled (Roberts and Stirling, 1971; Mackie, 1976), which suggests that impulses propagate from cell to cell by direct current flow. Even where direct evidence of coupling is lacking, the presence of gap junctions points to the same conclusion.

While hydrozoan conducting epithelia are typically either themselves contractile or provide a pathway for activation of a muscle (see Table I in Mackie and Passano, 1968), the siphonophore *Hippopodius* presents an unusual wealth of epithelially-mediated responses including, in addition to muscular involution, luminescence, blanching (temporary increases in opacity) and discharge of secretory material (Mackie, 1965; Mackie and Mackie, 1967; Mackie, 1976). This paper presents new results on these phenomena and their control. A general account of the anatomy and behavior will first be given.

Hippopodius is a colony consisting of a stem from which are budded a series of medusoid members, the nectophores, as well as other, polypoid, members

[1] Laboratoire d'Histologie et Biologie Tissulaire, Faculté des Sciences, 69621 Villeurbanne, France.

[2] Department of Biology, University of Victoria, British Columbia, Canada.

[3] Department of Zoology, University of Wisconsin, Madison, U.S.A.

FIGURE 1. Photograph of a living *Hippopodius*. The stem and appendages are partially retracted into the central space. The upper nectophores are more strongly blanched than the lower. The lowest nectophore (on the right) is transparent except for the marginal "prongs". Actual size of the colony is about 7 cm.

(Carré, 1968) ; the nectophores (also called nectocalyces) are the largest individuals and the only locomotory ones. The group of nectophores encloses a central space into which the stem and gastozooids can be withdrawn (Figs. 1, 2). There is no float, but the gelatinous mesogloea of the nectophores provides flotation (Jacobs, 1937).

The nectophores lack tentacles, sense organs, gonads and manubrium but resemble solitary hydromedusae in their locomotory neuromuscular organization.

Nutrients are conveyed to them by a canal from the stem which splits into branches upon entering the subumbrella. One of these, the ventral canal, is the site of an epithelial gland, the *rete mirabilis* or rete. Nectophores can swim using their circular, striated muscle, or roll up the margin (involution), using the radial system of smooth muscle fibers. Marginal nerve rings are present as in hydromedusae, but there is no general nerve plexus over the sub- or ex-umbrella and there are no nervous connections between the marginal nerve centers of the different nectophores in the colony (Mackie, 1965).

Considered as a medusoid, the nectophore is highly asymmetrical compared with free medusae (Figs. 1, 2, 14). The mesogloea is concentrated dorsally, while the subumbrella cavity is displaced ventrally. The outer or dorsal surface is a convex dome, the inner, ventral side being more concave. These features allow the nectophores to fit together into an interlocking structure with smooth sides and with the swimming jets directed backward. The exumbrella surface is generally

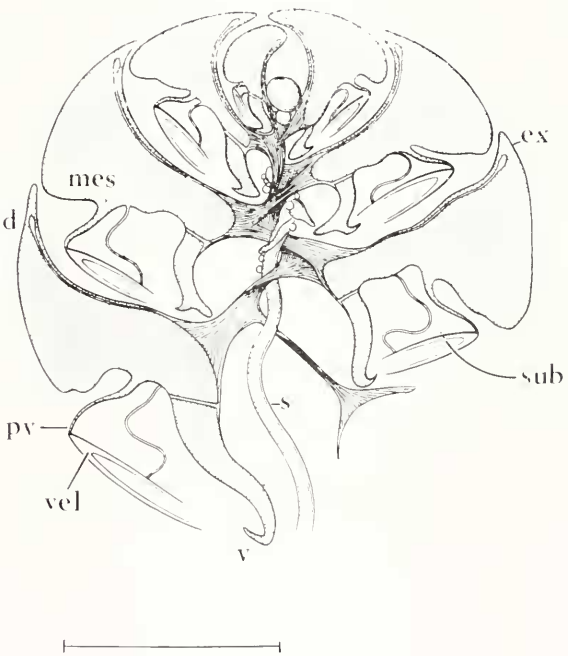

FIGURE 2. Internal morphology (after Chun, 1897). The "axis" of the colony is defined by the stem(s) which hangs in a central space surrounded by nectophores. Branched stem processes join the nectophores together, attaching to their axial surfaces. "Top" and "bottom" refer to aspects of the colony seen in its normal orientation, as shown here. "Dorsal" and "ventral" poles of the nectophore are indicated for the bottom nectophore on the left (d, v); the exumbrellar surface (ex) is the entire outer surface of the nectophore; the subumbrellar cavity (sub) is the inner chamber whose muscular walls produce the locomotory water jet. Other symbols: mes = mesogloea; pv = pseudovelum; vel = velum. Scale is 1 cm.

smooth except for a ridge bearing four prominent mounds ("prongs") encircling the upper rim of the subumbrellar cavity, and two ventral projections, or "horns".

The exumbrellar epithelium is a fragile, thin layer, one cell thick in all parts. It is thicker over the horns than elsewhere. It is partially syncytial (Mackie, 1965), but no physiological differences have been found between the syncytial and non-syncytial regions.

Freshly collected specimens of the siphonophore float or swim freely in the aquarium, but after a few hours most sink to the bottom. Jacobs (1937) suggests that they can regulate their buoyancy. All the nectophores make swimming movements but, except for the bottom two, which are responsible for locomotion, their activities do no more than create water movements within the center of the colony. As noted by D. A. Boag (cited by Mackie, 1964), swimming is not synchronized, although activity beginning in one nectophore may create a mechanical disturbance which causes others to become active. As in hydromedusae (Romanes, 1876; Passano, Mackie and Pavans de Ceccatty, 1967) the swimming rhythm is presumably independently generated by nervous pacemakers in the margin of each nectophore. Although there are no ocelli, *Hippopodius* aggregates in lighted regions of the tank (Mackie and Boag, 1963). This suggests that the pacemaker neurons controlling swimming are themselves photoreceptors, as in the jellyfish *Polyorchis* (Anderson and Mackie, 1977).

There are four, separate, direct effector responses to nectophore stimulation that are conducted through the colony, at least in part, by epithelial conduction (Mackie, 1965).

Involution of the margin (a response resembling "crumpling" in solitary hydromedusae) follows stimulation of any part of the exumbrellar surface; the epithelium is excitable, and conducts impulses to the margin where the radial muscles responsible for involution are located.

Undamaged specimens which have not recently been stimulated are transparent, but tactile or electrical stimulation causes the nectophore to become opaque; this "blanching" (Korotneff, 1884) is due to light-scattering granules which appear extracellularly in the mesogloea adjacent to the exumbrellar epithelium Kölliker, 1853) and are seen under the microscope as dense, spherical particles in the range 0.1 to 1.5 μm (Mackie and Mackie, 1967). Intact epithelium is necessary both for the spread of the blanching response and for the restoration of transparency, which occurs after a delay of several minutes. Mackie and Mackie (1967) proposed that impulses propagated in the epithelium trigger blanching. Tentative evidence was found for a role of Ca^{++} in the response and it was suggested that Ca^{++} activates the transformation of a mesogloeal protein between dispersed and aggregated configurations.

Seen in the dark, *Hippopodius* luminesces briefly when exposed to the same sorts of stimuli that provoke blanching and involution. Dubois (1914) has stated that this "magnificent bluish illumination" comes from the epithelial cells. Mackie and Mackie (1967) found that luminescence requires an intact exumbrellar epithelium and they proposed that photogenesis is intracellular and triggered by impulse conduction. Nicol (1958) obtained photometric records from *Hippopodius* and the closely related *Vogtia*. The chemical basis of the luminous reaction

remains unknown but, as reviewed by Morin and Hastings (1971); Cormier, Hori, Karkhanis, Anderson, Wampler, Morin and Hastings (1973); Anderson, Charbonneau and Cormier (1974); Morin (1974); and Blinks, Prendergast and Allen (1976), a calcium sensitive photoprotein like aequorin has been found in all bioluminescent coelenterates so far studied.

The fourth effector response associated with epithelial conduction occurs in the rete, a patch of giant endoderm cells clustered around ramifying branches of the ventral radial canal. The cells are specialized for protein secretion, but the chemical identity and biological role of the product remain uncertain. The rete cells are electrically coupled and connected by gap junctions. Impulses propagate through the tissue at about 10 cm/sec. When stimulated repetitively, the potentials associated with secretion sum and facilitate. The cells swell up and release secretion product; this process is Ca^{++}-dependent (Mackie, 1976).

These four responses tend to occur together under similar circumstances, for instance, when the nectophore surfaces are stimulated by abrupt contact. It has been suggested (Mackie and Mackie, 1967) that they have a common protective significance, the involution protecting the marginal locomotory centers, and luminescence and blanching serving to confuse, blind or alarm predators. The role of the secretion is not known but it may also be defensive. Spread of these responses through the colony accompanies colony-wide spread of epithelial impulses. An impulse may fail to spread beyond the bounds of a single nectophore but, with repetitive stimulation, all parts are rapidly invaded. At the same time, the stem is withdrawn, swimming ceases, and the colony remains in this defensive condition for periods up to several minutes, depending on the intensity with which it has been stimulated.

Materials and Methods

Specimens of the siphonophore *Hippopodius hippopus* Forskal were collected during spring, with a hand net, at the surface, in the bay of Villefranche sur Mer (France), where they occurred in the plankton with highly variable abundance from day to day. Stored at 15° C, in water changed daily, they remained healthy for a few days. Best maintenance was achieved when they were kept in freshly obtained, uncontaminated sea water.

Nectophores were isolated by cutting the stem which links them to the colony and placing them in a glass dish with flat, parallel sides and the bottom coated with a layer of transparent, polymerized resin (Sylgard 184, Dow Corning). They were immobilized either by pinning through the exumbrella or by restraining them within a corral of pins. Most of the records were made at room temperature (about 20° C) but, in some cases, a water-cooled microscope stage was used to keep the observation chamber at about 16° C. Before experiments involving stimulation of luminescence or blanching, the preparations were allowed to rest in darkness for at least 10 min.

The behavioral responses of the nectophores were analyzed using photometric procedures (Fig. 3) and records were made either on a storage oscilloscope (Tektronix 5015) or on chart recorders (Hewlett Packard 7402 A).

Bioluminescent emissions were detected by a RCA 1P21 photomultiplier (PM)

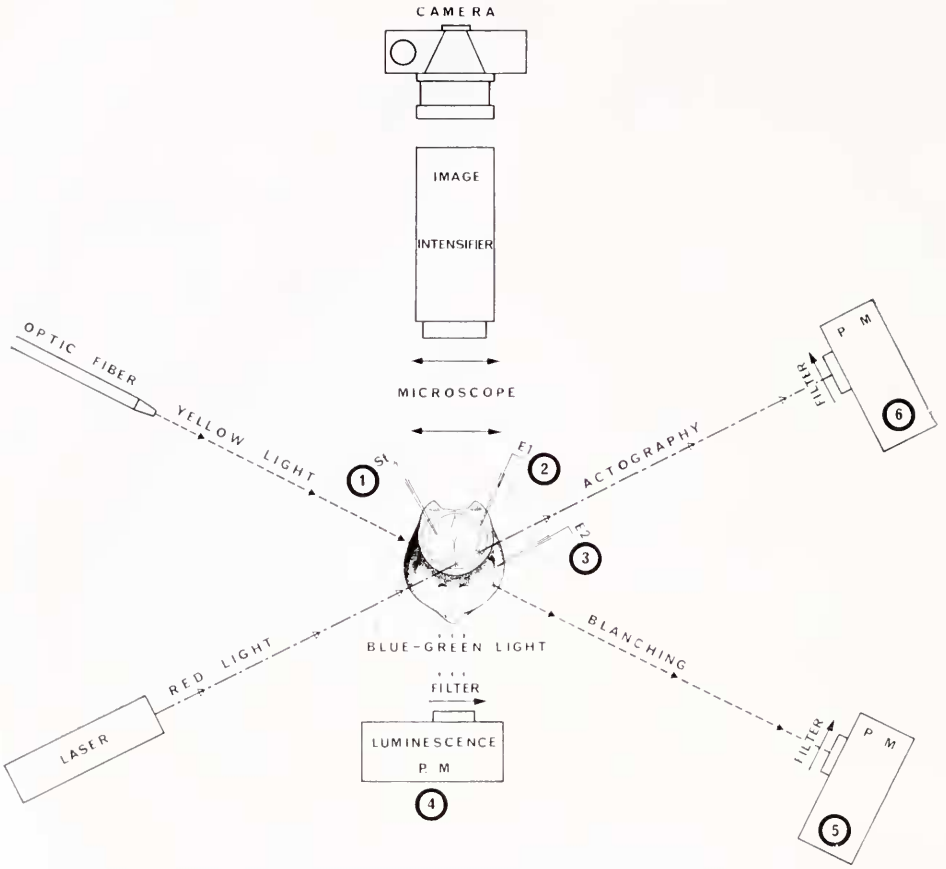

FIGURE 3. Schematic drawing of the experimental set up. Suction electrodes (1), (2), (3) allow stimulation and recording of electrical events. Luminescence is detected with a photomultiplier (4), while the sites of emission are observed or photographed through a microscope and image intensifier. A beam of yellow light crosses the exumbrella, and changes in transparency are measured with a photomultiplier (5). A beam of red light emitted by a laser crosses the velar region, making possible the detection of movements with a photomultiplier (6).

located below the preparation, and restricted to the blue range with a low pass filter (MTO 529b). In some preparations, luminescence was recorded from small areas of the surface of the animal, using one or two 1 mm diameter optic fibers leading to a EMI 9636 PM.

Actography was done by using a continuous laser (Spectra-Physics He-Ar, 1 mW). The red beam (6340 Å) of coherent light, attenuated by a netural filter (T: 20%), was set to cross the muscular ring of the pseudovelum. The diffraction image thus obtained was enlarged with a lens and delimited by a diaphragm so as to fit the diameter of the window of the PM, itself adjustable

with another diaphragm. A narrow band filter (MTO 7625a) restricted the PM sensitivity to the laser light only.

Changes in transparency have been recorded in some instances with this laser device, since blanching attenuates the light by diffusion. Nevertheless, a larger beam of normal (non-coherent) light proved to be more sensitive for blanching measurements, and it was less affected by movements. The light emerging from a light pipe, illuminated by a battery powered quartz-iodine lamp, was narrowed with a condenser lens to a beam of about 3 mm diameter and set to cross the exumbrella. An orange or yellow-green filter was used to obtain monochromatic light; an identical filter was placed on the window of the PM. It was thus possible to get simultaneous records of bioluminescence, blanching and muscular movements (Fig. 3).

Photography of the preparation by its own light was done through a Wild stereomicroscope. Owing to the brief duration and weakness of the light emission to be recorded, an image intensifier (Thomson CSF, three stages, gain: 50,000), was interposed in front of the camera. Its enhanced image was recorded on fast panchromatic (Ilford HP400) film; the exposure time, of 1 to 10 sec was recorded together with the photometric measurements. In some cases, the luminescent image was recorded with a TV camera and tape recorder (Sanyo VTC 7100) to allow frame by frame analysis of the flashes.

Electrical activities were generally recorded with suction electrodes made from drawn-out polyethylene catheter tubing and held by mechanical micromanipulators. The signals were amplified with Tektronix AC or DC high gain preamplifiers allowing frequency filtration. Mechanical stimulation of the nectophores was done by gently touching them with a fine plastic tube, such as a suction electrode. For electrical stimulation, monophasic pulses generated by a Grass S 44 stimulator were fed through an isolation unit to metal electrodes

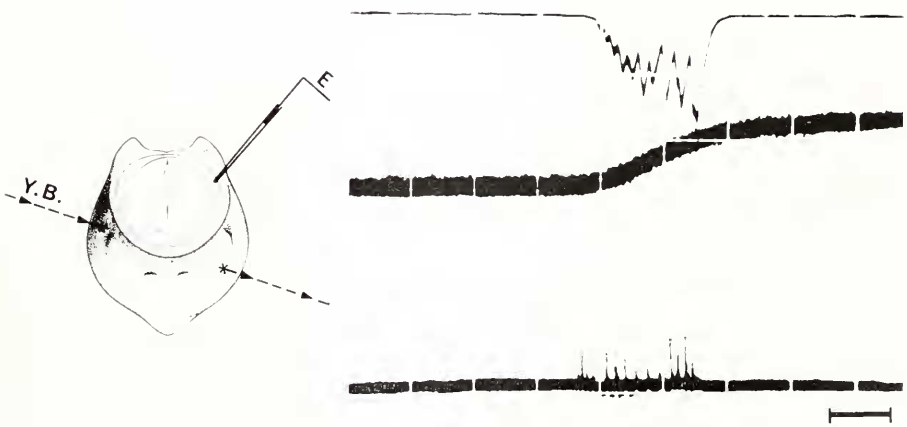

FIGURE 4. Spontaneous activities. Correlation of bioluminescence (upper line, downwards) and blanching (middle line, opacification upwards) with epithelial signals (lower line) recorded with electrode E on the velar region. Y.B. shows the beam of yellow light used to measure transparency. Scale is 2 sec.

or, more often, to a suction electrode attached to the surface of the animal or resting gently against it.

Experimental operations on the nectophores included surgical interventions such as ablation of the nerve ring, immobilization of a dissected piece of tissue held to the Sylgard base with very fine cactus spines, and cutting of parts of the nectophore. Fragments of nectophores were fixed for electron microscopy in 6% glutaraldehyde in a 0.4 M cacodylate buffer pH 7.4, embedded in epon, cut with a LKB microtome and observed with a Philips EM 300 microscope.

Results

Spontaneous events

Isolated nectophores remain capable of all the behavioral activities described in the colony. Usually, a nectophore which has been detached from the colony and immobilized in the observation chamber is opalescent (blanching having occurred during manipulations) and completely motionless. After 10 to 20 min, transparency is recovered again, except at the spots where pins have pierced the epithelium; the prongs and the horns are the last to lose their opacity.

The first "spontaneous" movement to be observed is an involution of the margin, followed by a slow relaxation. Then, other involutions may occur. Independently, isolated flashes or short flickering series of flashes are emitted (Fig. 4), again without any external stimulation. Combined records show blanching occuring together with each luminous event. A period of quietness follows, which usually lasts 10 to 20 min. Then swimming begins, in bursts of rhythmic activity alternating with periods of rest of 10 to 30 sec. The duration of swimming bouts steadily increases. The beating rhythm is very regular, about 4/sec at 20° C (Fig. 5). Our actographic technique, which measures changes in the diffraction pattern of the laser beam, has an extreme sensitivity towards minute displacements, but the shape and the amplitude of the curves are not significant; they change considerably according to the orientation of the beam. It is still noteworthy that under the same conditions, records of successive beats

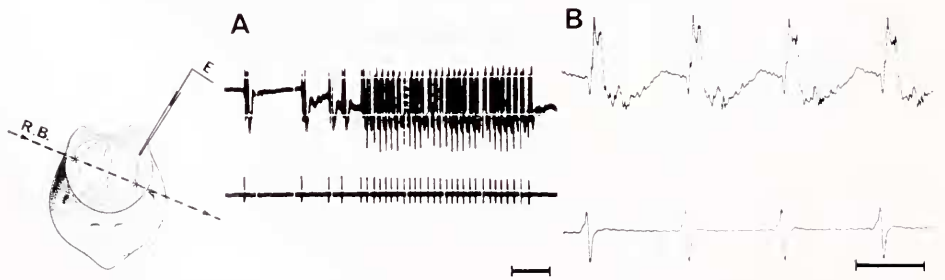

Figure 5. Swimming recorded actographically (upper line) and electrically from the velum (lower line). R.B. indicates the laser beam of red light used for actography; swimming typically begins irregularly and becomes rhythmic, ceasing abruptly. Scale is .5 sec for figure A and 200 msec for figure B.

are absolutely identical. Bioluminescence has never been observed during swimming.

Specific electrical signals are related to these activities. One type of potential is associated with involution. Another accompanies each swimming contraction (Fig. 5). Each flash is associated with a potential even in the case of multiple flashes where summation occurs (Fig. 4). But while the potentials associated with muscle contraction are best recorded on the marginal ring, those associated with bioluminescence spread over the whole exumbrellar surface and can be recorded on every part of it. Other spikes have been recorded on the nerve ring. They are without any obvious behavioral correlates, and show erratic patterns, consisting of isolated pulses or short bursts, the potentials usually being of small amplitude. Some of these events may be homologous to known pulses in hydromedusae (see Passano, 1973).

Effect of stimulation

It appears that the effector responses of the nectophore are organized within two general action systems, each with its own conduction pathways.

Involution, bioluminescence, blanching and secretion by the rete gland may result from mechanical or electrical stimulation applied *anywhere* on the surface of the exumbrellar epithelium. These stimuli never induce swimming; on the contrary, swimming stops when involution occurs.

The *swimming system* is independent. From the histological relationships and by analogy with solitary hydromesudae, it can be assumed that the swimming rhythm originates in the marginal nerve ring. Since there is no subumbrellar nerve net, transmission to the striated muscle sheet must occur locally in the vicinity of the nerve ring and conduction within the sheet must be myoid. Swimming is blocked by the addition of Mg^{++}, which presumably blocks the neuromuscular junctions. The circular muscles of the velum act together with those of the pseudovelum (the flexible margin of umbrella) and can be considered as a part of the same functional unit.

The other system involves epithelial conduction. Following stimulation any-

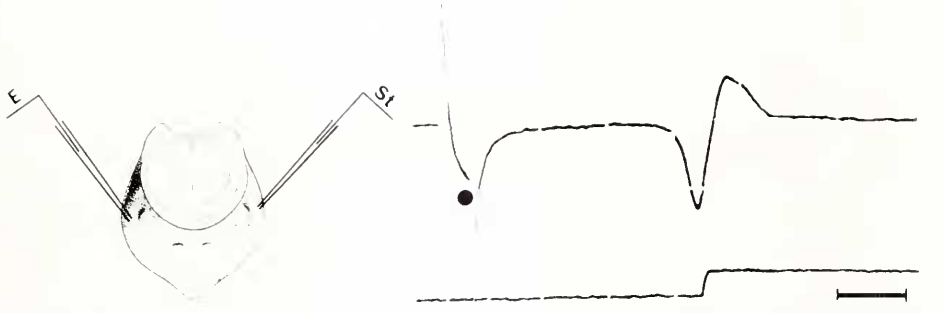

FIGURE 6. Epithelial potential recorded from the exumbrella with electrode E, after stimulation at St. The dot indicates the stimulation artifact. The amplitude calibration on the lower trace is 200 μv and the time scale 10 msec.

where on the exumbrella, a potential can be recorded at any place on the exumbrellar surface (Fig. 6). Its propagation is non-decremental; conduction time corresponds to the distance between stimulating and recording electrodes. The speed of propagation is appoximately 10 cm/sec at 22° C.

Involution

A single electrical shock above threshold leads to involution. The muscular components involved are two sets of smooth muscle fibers orientated radially, one in the velum and one in the subumbrellar endoderm of the pseudovelum (Fig. 7). Contraction of the first set of fibers causes the whole velum, which is like an iris diaphragm, to dilate and, seen in radial section, to curl outward. Contraction of the second set causes the pseudovelum to roll inward, carrying the curled velum within it. Impulses propagated through the exumbrellar epithelium normally cause both sets of muscles to contract in the involution response although, after repeated stimulation, one component may fail before the other (Fig. 8). The response amplitude is graded according to the number of impulses generated. Stimulation of the subumbrella is also effective.

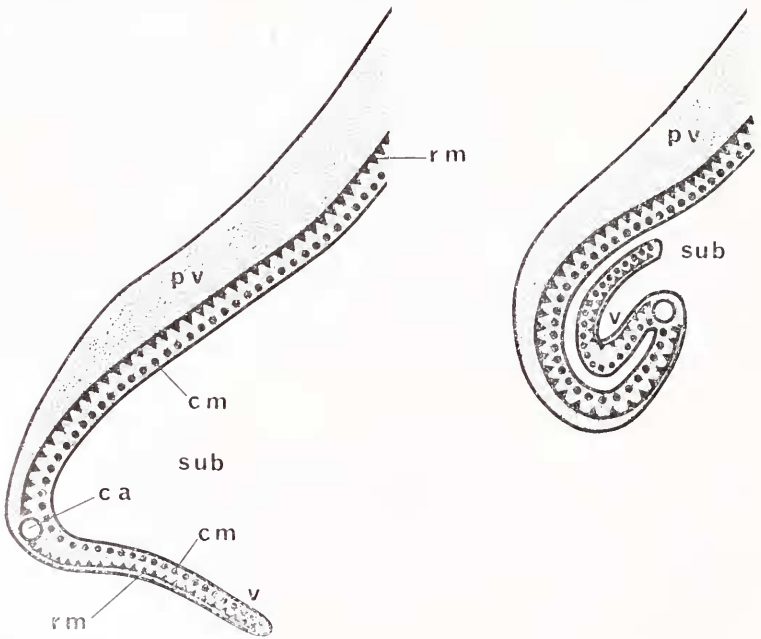

FIGURE 7. Schematic radial section through the contractile region of a nectophore, left at rest and right after involution. Involution is caused by the contraction of the two sets of radial muscle (rm) of the velum (v) and pseudovelum (pv), respectively. The circular muscle (cm) of the swimming system is not active during involution; ca represents the ring canal and sub the subumbrellar cavity.

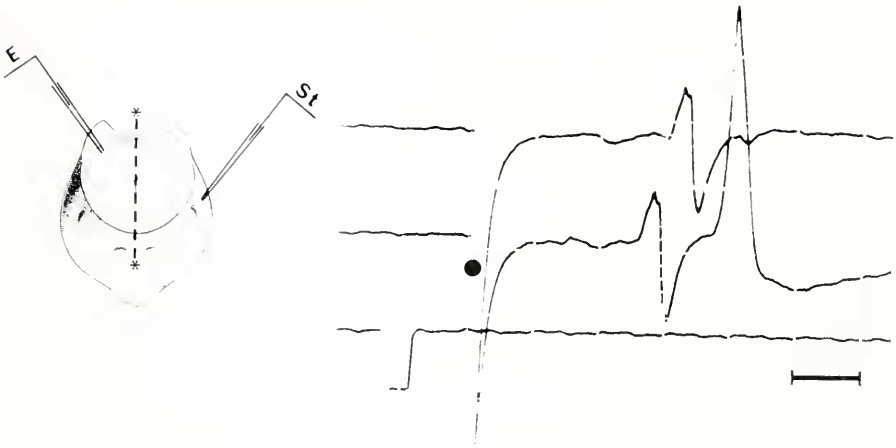

FIGURE 8. Experiment demonstrating the conduction across a "bridge" of exumbrellar epithelium. An incision (dashed line between asterisks) prevents conduction in the muscular regions. The experiment also illustrates the two components of involution. In the first trace, only the velar radial muscles responded. In the second trace, those of the pseudovelum responded immediately afterwards. The dot indicates the stimulation artefact; the lower line shows the 200 μv voltage calibration. Time scale is 10 msec.

Bioluminescence

"Ice blue" in color, often surprisingly strong, bioluminescence accompanies involution and, like it, is stimulated by mechanical or electrical shocks (Fig. 9). The basic response is a single flash, highly variable in duration (40–400 msec) and characterized by a fast ascending slope and a longer decay of the first order; double or multiple flashes also often occur and several flashes may lead to a rapid flickering post discharge (Fig. 13). Glow has never been observed.

Repetitive stimulation at 5 or 1 Hz, just above threshold, evokes one to one responses. Series which comprise 10 to 40 flashes show first a period of facilitation, followed by a more or less fluctuating plateau (Fig. 9). This may cease abruptly or decay gradually. For bioluminescence to be re-evoked, stronger stimulation or a period of rest is necessary.

One to one responses follow an acceleration of the stimulation frequency up to about 10 Hz. In addition to facilitation, partial summation is then apparent.

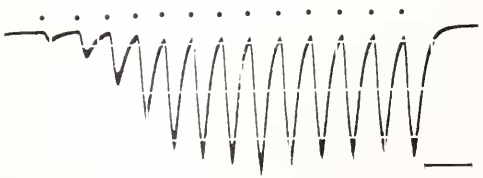

FIGURE 9. A series of shocks at lower frequency (dots) evokes a facilitating series of flashes. Scale is 1 sec.

Post discharges occur with stronger stimuli consisting of brief bursts at high frequency. When such stimuli are repeated, successive post discharges get longer and brighter, involving more and more cumulative flashes. They often lead to the climax of an explosive response, in which flashes flicker at high frequency (10 per second) for several seconds. After such intense events, which remind the "frenzy" responses observed by Buck (1973) in *Renilla,* the system is nearly exhausted. Nevertheless, after 5 to 20 min of recovery, the system is again responsive.

The effectiveness of electrical stimulation has proven to be highly variable in different individuals. This is probably due to the fragility of the epithelium which, if damaged by the electrodes, can be excited only by increased stimulation.

An epithelial potential is associated with each single flash as well as with each peak of a multiple response (Fig. 10). These pulses remain constant in shape and amplitude regardless of the brightness of the associated flash. Response latencies vary depending on the placement of the electrodes.

Initially, at least three consecutive epithelial pulses are required to elicit the first component of the luminous response (Fig. 10). Thereafter, one to one responses occur when the stimulating shocks, and thus the epithelial pulses, are

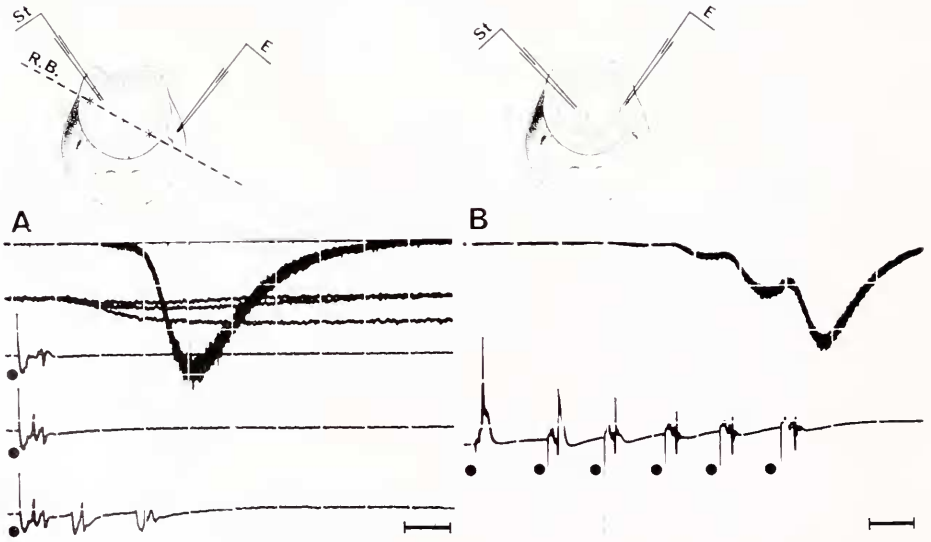

FIGURE 10. Induction of luminescence. On left, A is a simultaneous record of luminescence (upper line), involution (actographic record, next below) and epithelial impulses (three lower records, successive sweeps about 4 sec apart). Single epithelial impulses produced perceptible degrees of involution, but no flash occurred until three epithelial pulses were evoked. Scale is 50 msec. On right, in B, a series of shocks evoked a corresponding series of epithelial pulses (lower trace). After the third one luminescence was evoked; each subsequent spike was associated with a peak of the luminous response. Summation and facilitation are apparent; the latencies are not significant; amplitude changes in the recorded electrical signal are due to movement of the tissue with respect to the tip of the recording electrode. Scale is 0.2 sec.

separated by less than 3 to 5 sec. Otherwise, two or three pulses are needed before again obtaining a luminous response.

The post discharge volley of epithelial pulses, as well as corresponding flashes observed after intense stimulation, often show an inherent rhythmicity.

Sites of emission

Although the transparency of the nectophore and the shortness of the flashes make observation difficult, it appears clear that the light arises from the most external layer of the exumbrella. The velum, the subumbrellar surface and internal structures such as the endodermal canals crossing the mesogloea never luminesce. Removal of part of the exumbrellar epithelium results in loss of the ability of this region to luminesce.

Nevertheless, the exumbrellar surface does not respond as a whole to stimulation, whatever the placement of the electrodes. What is more, the active area moves its boundaries from one flash to the next. Usually, the response originates in the horns. Successive flashes of increasing intensity extend the active territory along two symmetrical, curved and narrow paths, which soon fuse, forming a sort of horseshoe around the margin. Then, with further flashes, the active territory moves toward the bell-shaped dorsal face of the nectophore. Illumination is then particularly strong beside the horns and around the marginal prongs. The apex of the dome is invaded only during paroxysmic responses. With the last flashes, which become weaker and weaker, the active territory either shrinks quickly to the originally active zones or moves to parts of the horseshoe region which had not been fully exploited previously. Photographic records through the image intensifier (Figs. 11, 12) required the integration of several successive flashes for correct exposure. Nevertheless, moving boundaries and increase of active surface are well demonstrated in correlation with the facilitation which occurs during successive series of responses.

Observed during a single flash, the active area, whatever its localization and dimensions, gives the impression of being illuminated by a wave. In the case of a rapid succession of flashes, this wave rebounds and whirls, often switching alternatively to the left and the right sides of the nectophore. This asynchronous illumination of the area of activity is clearly related to the duration of the flashes and their waveform.

Blanching

Blanching is also a property of the exumbrella, but involves both the epithelium and the underlying cortical layer of the mesogloea, where the light-scattering granules actually appear.

Blanching first appears as a local response at the point of application of the electrical or mechanical stimulus, then spreads over the whole exumbrella as a wave. The first generalized response of opacification is the strongest one. It takes up to 10 sec to build exponentially to a stable level. With further shocks, blanching responses get smaller and smaller, apparently reaching exponentially the maximum level of opacification.

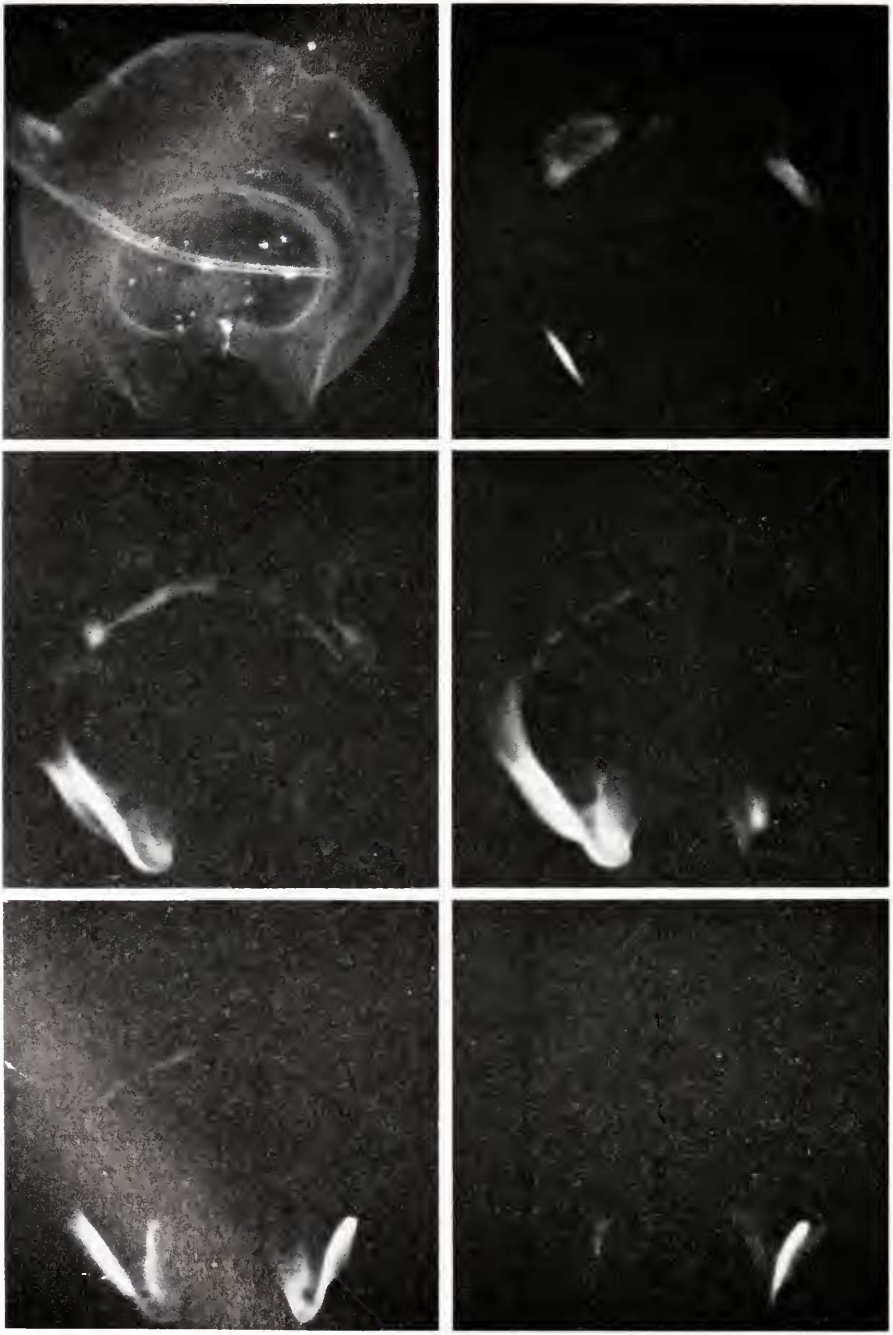

FIGURE 11. Autophotographic pictures of a nectophore during successive luminous emissions, taken through microscope and image intensifier. The first picture shows the nectophore

Deblanching occurs progressively over a period of 10 min to 1 hr according to the degree of opacity at the start. The horns are the last to clear and often remain opaque while the rest of the exumbrella has cleared completely.

Blanching and bioluminescence are evoked together by electrical stimulation. The records show that the time course of the two events may correspond closely, or one may be slightly delayed (Fig. 13). The first generalized blanching response can precede the first flash, but the minimum number of impulses needed to evoke blanching has not been ascertained. Blanching builds up to its saturation level long before the exhaustion of luminescence.

The conduction pathways

Figure 14 shows one of a number of preparations used to explore conduction routes in the nectophore. After an incision which separates the marginal ring from the exumbrellar ectoderm (operation A) impulses evoked at St1 or St2, on the ring, propagate to the radial muscles of the velum and also to those of the pseudovelum. Since the latter lie in the endoderm, and since there are no alternative routes by which impulses could enter the endoderm, it is assumed that they enter at some point around the margin. Direct connections between ectoderm and endoderm occur in this location in another siphonophore, *Nanomia* (C. L. Singla, personal communication).

Stimulation on the exumbrellar epithelium at St3 fails to excite the velar muscle but the pseudovelar fibers contract. This suggests that impulses evoked in the exumbrellar ectoderm can pass around to the back of the nectrophore, enter the axial endoderm canal and travel through it to the subumbrellar side. They do not then pass out into the ectoderm again, showing that conduction between the two layers in this region is polarized inward.

Confirmation that the axial endoderm canal is the conduction route in the previous experiment comes from adding the destruction of the axial canal (operation B) to a nectophore already bearing the incision A. There is no change in the response following stimulation at St1, but stimulation at St3 now fails to excite the endodermal radial muscles.

Stimulation on the exumbrellar surface at S3 in an intact, unoperated nectophore evokes contraction first of the pseudovelar muscle, then, after a long delay, that of the velum (Figs. 7, 8). Presumably impulses have had to pass out via the axial canal to the back and then around to the front again via the ectoderm. After operation A or B this pathway is blocked, and only the pseudovelum responds (Fig. 15).

These experiments point unequivocally to the existence of conduction bridges between the ectoderm and the endoderm at two points, one at the margin and one

---

in dim artificial light; the suction electrode used for electrical stimulation ends in the pseudovelum region. The following pictures (from left to right and high to low), integrate about ten flashes each, provoked by repetitive stimulation, as in Figure 9. The successive zones of luminescent activity are clearly shown, the strongest responses being given in the horns and at the base of the prongs. Activity can switch from one region to another. Nectophore diameter is approximately 1.5 cm.

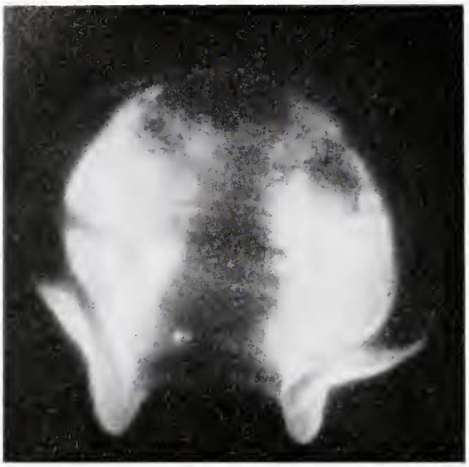

FIGURE 12. Autophotographic pictures of another nectophore during a paroxysmic response. Luminescence spreads from the horns to the back of the nectophore in successive pulsating waves, thus revealing most of the exumbrellar surface in this picture exposed several seconds. Nectophore diameter is approximately 1.5 cm.

at the back of the nectophore. These bridges have not yet been located histologically in *Hippopodius*, but conducting bridges of the sort proposed occur in hydromedusae at one of these locations, the margin (Mackie and Passano, 1968).

It follows from these observations that in the normal, intact nectophore, stimulation of either the exumbrella or subumbrella will give rise to impulses

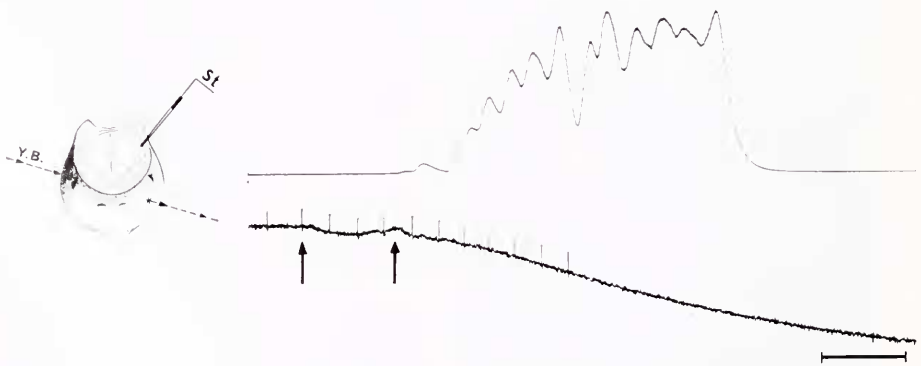

FIGURE 13. Correlation of involution, luminescence and blanching. A series of electrical shocks, shown superimposed on the blanching record (lower line, opacification downwards) evoked a multiple luminescent response (upper record, relative intensity increasing upwards) as well as involution and blanching. Involution, which occurs first, is detected as a slight deflection (between arrows). Blanching, detected by the attenuation of the yellow beam of light (Y.B.) still progresses after the end of the luminous response. Scale is 1 sec.

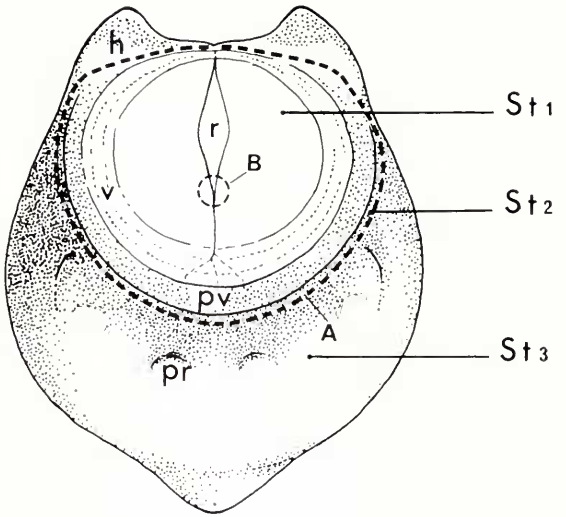

FIGURE 14. Conduction routes in the nectophore. All stimulating points (St) and cuts (dotted lines) are on the front (abaxial) side of the nectophore. Cut A is an incision through the exumbrellar ectoderm around the marginal ring separating the ectoderm within the ring, on which point St1 and St2 lie, from that outside, where St3 lies. Cut B destroys the axial canal at first point of entry into the subumbrella, separating the subumbrellar endoderm from the endoderm of the axial side. A shock at point St1 on the subumbrellar surface stimulates both the ectoderm and the endodermal lamella which lies close beneath it. This ectoderm is inexcitable, however, so the effect is simply that of exciting the endoderm. The experimental results are described in the text (h represents a horn; pr: prong; pv: pseudovelum; r: rete; v: velum).

that invade both areas. The polarization of ecto-endodermal conduction at the margin may therefore have no functional significance, since impulses have another route by which to pass to the exumbrella from the subumbrella.

Conduction between the nectophore and stem does not appear to involve direct epithelial pathways. A "translation" step intervenes, by which epithelial signals excite nervous activity in the stem tissue at its junction with the nectophore. Likewise, nervous activity in the stem appears to be translated into epithelial impulses in the nectophore. These epithelio-neural and neuro-epithelial transfer steps do not operate on a one-for-one basis, and may require facilitation. Details of these interactions will be presented elsewhere (D. Carré and G. O. Mackie, unpublished).

Morphological data

An electron microscopic survey of the conducting and contracting tissues has been carried out in order to verify previous histological accounts, and to extend them particularly with regard to junctional structure (Fig. 16).

The exumbrellar epithelium is a conventional cellular layer on the axial surface near the stem attachment, but most other areas of the epithelium are

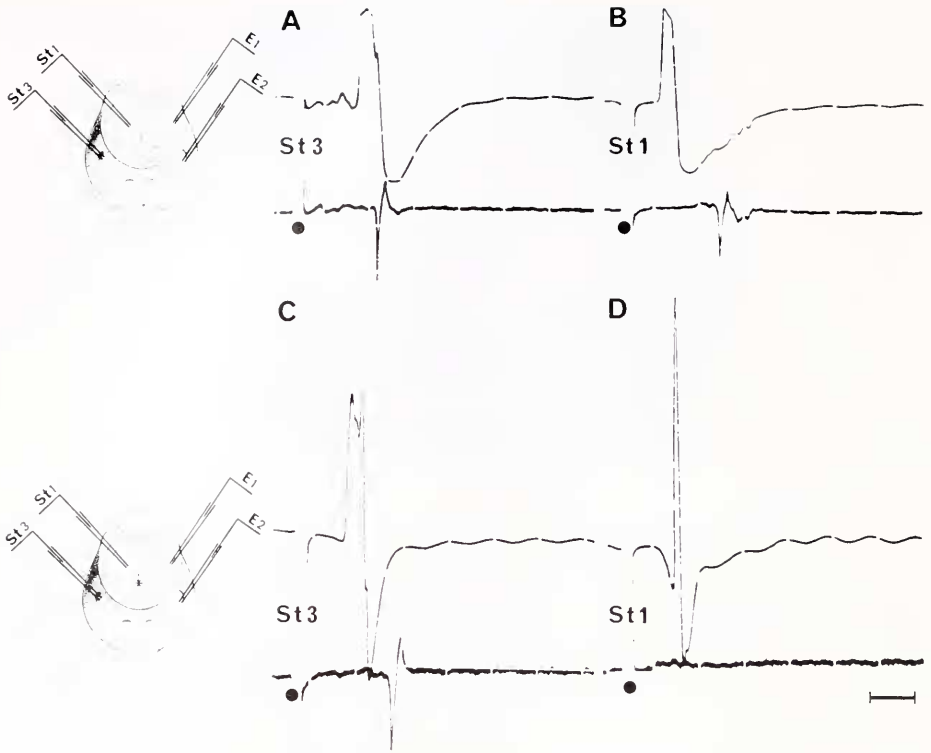

FIGURE 15. Conduction routes in the nectophore. Figures A and B are in an intact nectophore. Recording electrodes on the pseudovelum (E_1 and upper trace) and exumbrella (E_2 and lower trace) show contraction of the endodermal radial muscles and epithelial impulses, respectively. Stimuli were given on the exumbrella at St3 (Fig. A) or subumbrella at St1 (Fig. B). After destruction of the canal (operation B) excitation can still spread from ectoderm to endoderm (Fig. C), but not in the reverse direction (Fig. D). Scale is 20 msec.

syncytial (Iwantzoff, 1928; Mackie, 1965). Between cellular and syncytial zones a broader area consisting of multinucleate cells occurs. In the cellular regions, the adjacent membranes are connected by septate and gap junctions, resembling those of other hydrozoans, *e.g., Hydra* (Hand and Gobel, 1972) in their appearance and location (Figs. 11a, b). Physiologically, the whole epithelium acts as a syncytium and the membrane barriers offer no impediment to impulse spread. Gap junctions may be presumed to act as pathways for intercellular coupling (Gilula, 1973; Sheridan, 1973), while septate junctions probably act as diffusion barriers like tight junctions in vertebrates (Staehelin, 1974).

The exumbrellar cells contain no structures obviously associated with luminescence. Secretion grains occur (Fig. 11a), but similar grains occur in non luminous siphonophores (Mackie, 1965; Carré, 1975). The mesogloeal layer adjacent to the exumbrellar epithelium contains the blanching granules described previously (Mackie and Mackie, 1967) and is separated from the epithelium by a basement membrane (Fig. 16a) which is more prominent than the one found in

equivalent locations in non-blanching siphonophores, and might therefore be an important structure in the mediation of this response.

Electron microscopy of the subumbrella confirms the existence of two layers of muscles, the circular striated fibers of the ectoderm and the radial, smooth fibers of the endoderm (Fig. 16c). Gap junctions connect the cells in both layers (Fig. 16d, e). Nerves have not been seen in any part of the exumbrellar epithelium nor in the muscle layers.

Discussion

The exumbrellar epithelium of the nectophore of *Hippopodius* is an anatomically well-defined layer having a large surface area and some unusual properties. Functionally, the epithelium is able to transduce external stimuli, such as mechanical or electrical shocks, into action potentials, and to propagate them over the whole surface in an unpolarized and non-decremental way. These signals induce the contraction of the radial muscle fibers localized at the margin, causing involution and cessation of rhythmic swimming. Upon spreading to the endoderm they can induce secretion by the glandular cells of the rete. They can induce, within the epithelium itself, a fast bioluminescent response, often repeated rhythmically. Finally, they can induce a long lasting blanching response in the mesoglea adjacent to the epithelium.

In its aspects of sensory transduction and conduction leading to muscle contraction, the exumbrellar epithelium of *Hippopodius* behaves like several other known examples of conducting epithelia, particularly in medusae (Mackie and Passano, 1968). The large area of the exumbrellar sheet, much of it lying at a considerable distance from the nervous ring, makes these functions easy to demonstrate. As shown by the experiments summarized in Figures 14 and 15, impulses reach the velum radial fibers directly, and can cross to the endoderm at the margin by some route which has not yet been determined. They cannot, however, cross in the reverse direction. In the normal animal, ectodermal impulses will always lead to excitation of the endoderm, since they can enter the endoderm either at the margin or at the back of the nectophore, passing into the axial canal. The two-stage involution is a direct response to the epithelial pulses, which also block endogenous swimming for an extended period. The same pulses cause secretion in the rete gland (Mackie, 1976). The other effector responses, bioluminescence and blanching, call for more extensive comment.

Bioluminescence in *Hippopodius* occurs as a surface phenomenon in a wide, flat epithelium which is also the conducting pathway for the response. The preparation should, therefore, be useful for the correlation of the temporal and spatial aspects of the bioluminescent response.

The basic photometric manifestation is a flash. Rhythmic series of flashes are characterized by facilitation, followed by fatigue. And this rhythmic flashing, often summating and ending in an "explosive" response, can be autonomous. These features are known in several bioluminescent systems, such as, for example, the octocorallians *Renilla* (Buck, 1973) and *Veretillum* (Bilbaut, 1975a, b) the hydrozoan *Obelia* (Morin and Cooke 1971a, b) or the scale worm *Acholoe* (Bilbaut and Bassot, 1977). They lead to the distinction of several problems of

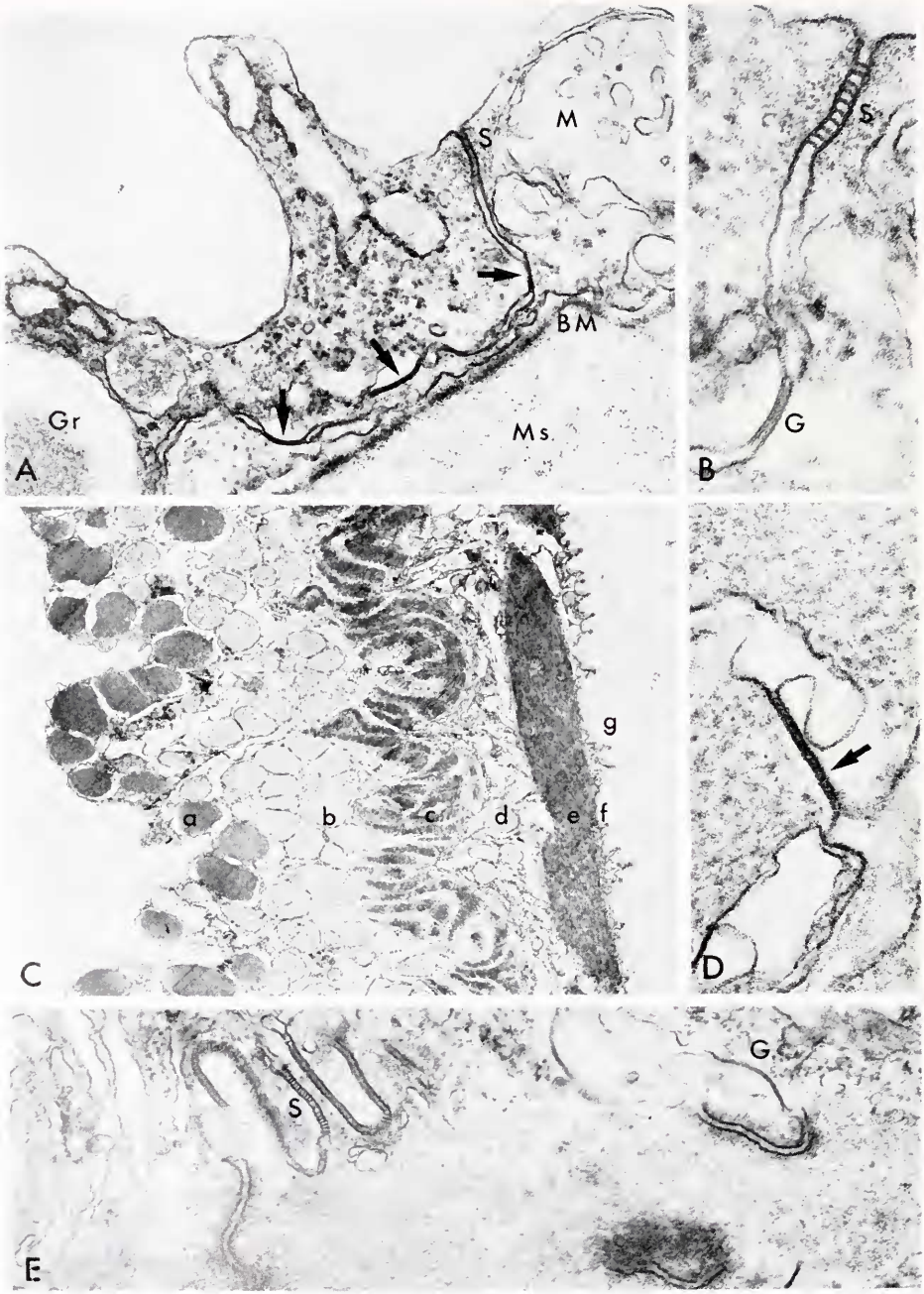

FIGURE 16. *A*. Junctional complex between two ectoderm cells of the axial cellular region of the exumbrella. The complex comprises a septate junction (S) close to the epithelial surface and several gap junctions (arrows) in the deep parts of junctional infoldings. Other abbreviations are: BM = basement membrane; Gr = secretion granule; M =

control: the excitation-bioluminescence coupling, the autoexcitation process, and the recruitment of new units of activity.

In *Hippopodius* the flash is always triggered by an epithelial potential; but the flash itself is not an electrogenic phenomenon, at least in our recording conditions: flash-inducing potentials remain the same regardless of the amplitude and the duration of the response. Since three successive pulses are needed to provoke a first flash, one may presume that the first two "prime" the mechanism of reactivity. In *Obelia* (Morin and Cooke, 1971a, b) an electrical potential is also associated with each flash, except the first one. If the initial stimulation is strong enough, autonomous rhythmic flashing occurs. It seems to represent a pacemaker like after-discharge. This manifestation recalls the "frenzy" autoexcitation state of *Renilla* during which luminous waves are propagated over the radial surface for up to an hour (Buck, 1973). However, in this case the polyps are linked by a nerve net which is assumed to initiate their luminous responses. The nerve free epithelium of *Hippopodius* provides a clear example of repetitive firing, a characteristic known in several epithelial conducting systems (Mackie and Passano, 1968).

Regarding localization, *Hippopodius* is not the first coelenterate examined with image intensification (Reynolds, 1972; Freeman and Reynolds, 1973; Buck, 1973; Morin and Reynolds, 1974) but, with its large emitting surface, it proves to be particularly interesting. Observations during flashes have shown three significant facts: first, the exumbrellar surface luminesces only in restricted area; secondly, within an active area, luminescence spreads like a wave during the flash; and thirdly, from one response to the next, the active zone changes its boundaries or even jumps from one place to another. It is thus clear that the temporal parameters of the flashes are determined by spatial features of the emitting surface. One can assume that the shape of the first responses, which originate in a rather small area within which they are nearly synchronous, comes close to illustrating the fundamental kinetics of the bioluminescent reaction. Successive flashes will increase in amplitude because of a wider surface of emission, and in duration because of the longer propagation distances involved. Double or multiple flashes presumably occur when several active zones, more or less separate or overlapping, are brought into action. The decrease in intensity at the end of the emission would be due partly to diminishing photogenic reserves, partly to a topographical retraction of the active zone which, step by step, returns to its initial boundaries, and partly to a decline in the frequency of the conducting impulses.

---

mitochondrion; Ms = mesogloea. *B*. Details of sepate (S) and gap (G) junctions. *C*. Radial section through the muscular tissues lining the pseudovelum. The secretion shows, from left to right: a) secretory granules at the surface of the ectoderm, b) areas of massed mitochondria in the ectodermal cells; c) striated muscle fibers lying at the base of the ectodermal layer, d) clusters of mitochondria in the endodermal layer, e) radial smooth muscle fibers of the endoderm, f) basement membrane, g) mesogloea. In this section nonnucleus is seen either in the ectodermal or in the endodermal cells. The mesogloea separating the two epithelia is a very thin layer next to the striated muscle fibers. *D*. Gap junction (arrow) between two ectodermal striated muscle fibers. *E*. Long and sinuous junctional complex between the ends of two endodermal smooth muscle fibers. Septate junctions (S) are more conspicuous than gap junctions (G).

The fundamentally progressive facilitation of the series of flashes requires a simple mechanism to account for the extension of the active zone. The active zone and the nonresponsive zone both receive the same epithelial pulses which spread all over the epithelium. One has thus to consider that the potentially photogenic units have to be "primed" before being reactive. This priming could be due to the repetition of the epithelial pulse, as a continuation of the phenomenon observed with the first flash occurring with the third epithelial pulse. It could be due to the spread of a modification resulting from the luminous reaction itself, such as a sequestration of calcium. As established on giant cells of dipteran salivary glands (Loewenstein, 1966; Rose and Loewenstein, 1976), the intracellular free Ca^{++} concentration regulates the opening of gap junctions. The priming mechanism could also relate to the existence of different territories in the exumbrellar epithelium. The occurrence of syncytial areas (Mackie, 1965) and of different thickness may be significant in this respect.

The visible spread of ignition within the active zone which occurs during a single flash is obviously different from the overall territorial extension. The ignition wave seems able to follow the boundaries of the active zone, to rebound and to circle in whorls in such a way that every part of the active zone is explored. This recalls again the spread of luminous waves in *Renilla* (Buck, 1973).

A set of closely similar observations have been made in a recent study of the sites of activity in the elytra during luminescence of scale-worms (Pavans de Ceccatty, Bassot, Bllbaut and Nicolas, 1977; Bilbaut and Bassot, 1977; Bassot and Bilbaut, 1977a, b). Elytra also have a photogenic epithelium which can be considered as a two-dimensional sheet; isolated elytra emit flashes, and rhythmic series of flashes are characterized by important and progressive variations in flash intensity, duration, delay, and frequency. These variations are also due to changes within a limited active zone and to step by step displacements of the zone. Thus, a similar mechanism of modulation related to the optional properties of the effector itself seems to be involved.

A major advantage of the elytra system is the precise identification of the morphological components. Luminescence, as well as related fluorescence, originates from photosomes, which are cytoplasmic paracrystals of endoplasmic reticulum. In *Hippopodius* the units of activity remain unknown. Their identification *in vivo* as cells, cluster of cells or subcellular components would require microscopic observation at high magnification during a flash. On sections, nothing comparable to "lumisomes" (Anderson and Cormier, 1973) or luminelles (Spurlock and Cormier, 1975) has been seen; the secretory granules observed in the epithelial cells are not yet significant enough. Nevertheless, bioluminescence is indeed a property of the epithelial cells and it seems to occur independently of any secretory discharge process.

Blanching and bioluminescence occur concomitantly only because they are responses to the same stimuli, and do not otherwise have any direct relationship. Blanching requires several seconds to reach its peak and has a considerably longer time-course than flashing. It quickly reaches a saturation level, after which further stimulation is ineffective, while luminescence can be evoked repeatedly over a long series of flashes. The nature of the reaction involving, again, calcium

(Mackie and Mackie, 1967), by which the blanching granules become visible and invisible, still requires elucidation.

In conclusion, the large epithelial surface of the exumbrella appears to play an essential role in the control of all the behavioral manifestations which follow an external stimulus. Swimming is an independent system functioning spontaneously, in rhythmic bursts, after a long period free from stimulation. A mechanical or electrical shock on the exumbrella inhibits the swmimming system and induces an involution of the velum and pseudovelum. Further stimulation reinforces this response and leads to dramatic changes in the appearance of the animal, normally transparent almost to the point of invisibility: blanching by daylight and bioluminescence by night make the siphonophore suddenly visible. The observation that flashing can be repetitive, and often rhythmic, implies control by a pacemaker system. But while epithelial pulses spread everywhere over the exumbrellar surface, the effector reacts only in limited areas. Displacements and configurational changes of the active sites of light emission during successive responses are at least partially responsible for the temporal variations in the wave form of the response measured photometrically. Since triggering events in the conducting system propagate on an all or nothing basis, it follows that facilitation of the luminescent response must be due primarly to an optional property of the effector itself, which allows an increase in the number of reactive units within a population of potentially photogenic units, the remainder of which are not yet primed to react to the epithelial pulse. With this mechanism, the photogenic effector would acquire the ability to produce repeated flashes from a given photogenic reserve, and to vary successive responses.

This work was supported by a NATO Grant No. 937. The participation of G. O. Mackie was made possible under the scientific exchange program between Canada and France. We gratefully acknowldge the use of the facilities of the Station Zoologique at Villefranche sur Mer.

Summary

1. Four responses are spread by through-conducting excitable epithelia in the nectophores: luminescence, blanching, muscular involution, and secretion. Swimming, which is independently controlled by the nervous system, is inhibited by epithelial impulses.

2. Luminescent flashes are correlated one for one with epithelial impulses. At least three impulses must be propagated before the first flash is recorded. Flashes sum and facilitate. Pacemaker-like after-discharges may continue after stimulation has ceased. Not all regions of the epithelium luminesce equally, and the active area can shift during a single luminescent episode, although the excitatory impulses pass across all regions equally. No steady luminescent glow has been observed. Comparisons are drawn with other luminescent systems.

3. Luminescence is generated intracellularly within the exumbrellar epithelium, but blanching (opacity) is associated with formation of granules in the adjacent mesogloea. The response builds up to saturation level within 10 sec, long before

the exhaustion of luminescence. Fading is gradual, transparency returning within an hour. Some regions blanch more strongly and fade more slowly than others.

4. Involution of the margin involves contraction of radial muscle fibres in the velar ectoderm and subumbrellar endoderm. The two groups are functionally coupled, but coupling may break down with repeated stimulation. Excitation can pass from ecto- to endoderm at the margin and either way between the two layers on the axial side of the nectophore. Thus, the endodermal effectors, including the secretory epithelium, are excited concurrently with the ectodermal.

5. Impulse conduction in the excitable epithelia and myoepithelia is assumed to involve electrical coupling mediated by gap junctions, which have been found by electron microscopy in the regions concerned.

LITERATURE CITED

Anderson, J. A., H. Charbonneau, and M. J. Cormier, 1974. Mechanism of calcium induction of *Renilla* bioluminescence. Involvement of a calcium-triggered luciferin binding protein. *Biochemistry,* **13**: 1195–1200.

Anderson, J. M., and M. J. Cormier, 1973. Lumisomes: the cellular site of bioluminescence in coelenterates. *J. Biol. Chem.,* **248**: 2937–2943.

Anderson, P. A. V., and G. O. Mackie, 1977. Electrically coupled photosensitive neurons control swimming in a jellyfish. *Science,* **197**: 186–188.

Bassot, J. M., and A. Bilbaut, 1977a. Bioluminescence des élytres d'Acholoe. III: Déplacement des sites d'origine au cours des émissions. *Biol. Cellulaire,* **28**: 155–162.

Bassot, J. M., and A. Bilbaut, 1977b. Bioluminescence des élytres d'Acholoe. IV: Luminescence et fluorescence des photosomes. *Biol. Cellulaire,* **28**: 163–168.

Bilbaut, A., 1975a. Etude de la bioluminescence chez l'octocoralliaire *Veretillum cynomorium.* I. Les réponses lumineuses des autozoïdes isolés de la colonie. *Arch. Zool. Exp. Gen.,* **116**: 27–42.

Bilbaut, A., 1975b. Etude de la bioluminescence chez l'octocoralliaire *Veretillum cynomorium.* II.: Les réponses lumineuses de la colonie et les réactions motrices associées. *Arch. Zool. Exp. Gen.,* **116**: 321–341.

Bilbaut, A., and J. M. Bassot, 1977. Bioluminescence des élytres d'Acholoe. II: Données photométriques. *Biol. Cellulaire,* **28**: 145–154.

Blinks, J. R., F. G. Prendergast, and D. G. Allen, 1976. Photoproteins as biological calcium indicators. *Pharmacol. Rev.,* **28**(1): 1–93.

Bone, Q., and G. O. Mackie, 1975. Skin impulses and locomotion in *Oikopleura* (Tunicata: Larvacea). *Biol. Bull.,* **149**: 267–286.

Buck, J., 1973. Bioluminescent behavior in *Renilla.* I: colonial responses. *Biol. Bull.,* **144**: 19–42.

Carré, D., 1968. Sur le développement post-larvaire d'*Hippopodius hippopus* (Forskal). *Cah. Biol. Mar.,* **9**: 417–420.

Carré, D., 1975. Contribution à l'étude des siphonophores. Embryologie, cnidogénèse, supports morphologiques de l'intégration. Thèse, Université de Paris VI, Station Zoologique, Villefranche sur Mer., 200 pp. (approx.).

Chun, C., 1897. Über den Bau und die morphologische Auffassung der Siphonophoren. *Verh. Dtsch Zool. Ges.,* **7**: 48–111.

Cormier, M. J., K. Hori, Y. D. Karkhanis, J. M. Anderson, J. E. Wampler, J. G. Morin, and J. W. Hastings, 1973. Evidence for similar biochemical requirements for bioluminescence among the Coelenterates. *J. Cell. Physiol.,* **81**: 291–298.

Dubois, R., 1914. *La vie et la lumière.* Paris, 348 pp. (cited in Harvey, N., *Bioluminescence.* Academic Press, 1952).

Freeman, G., and G. T. Reynolds, 1973. The development of bioluminescence in the Ctenophore *Mnemiopsis leidyi. Dev. Biol.,* **31**: 61–100.

Gilula, N. B., 1973. Development of cell junctions. *Am. Zool.,* **13**: 1109–1117.

HAND, A. R., AND S. GOBEL, 1972. The structural organization of the septate and gap junctions of *Hydra*. *J. Cell. Biol.*, **52**: 397–408.

IWANTZOFF, N. A., 1928. Beiträge zur Kenntnis der Histologie der Siphonophoren. *Bull. Soc. Nat. Moscou*, **37**: 1–36.

JACOBS, W., 1937. Beobachtungen über das Schweben der Siphonophoren. *Z. Vgl. Physiol.*, **24**: 583–601.

KÖLLIKER, A., 1853. Die Schwimmpolypen oder Siphonophoren von Messina, Leipzig (Engelman), 96 pp.

KOROTNEFF, A., 1884. Zur Histologie der Siphonophoren. *Mitt. Zool. Sta. Neapel*, **5**: 229–288.

LOEWENSTEIN, W. R., 1966. Permeability of membrane junctions. *Ann. N. Y. Acad. Sci.*, **137**: 441–472.

MACKIE, G. O., 1964. Analysis of locomotion in a siphonophore colony. *Proc. R. Soc. Lond. B. Biol. Sci.*, **159**: 366–391.

MACKIE, G. O., 1965. Conduction in the nerve-free epithelia of siphonophores. *Am. Zool.*, **5**: 439–453.

MACKIE, G. O., 1970. Neuroid conduction and the evolution of conducting tissues. *Q. Rev. Biol.*, **45**: 319–332.

MACKIE, G. O., 1976. Propagated spikes and secretion in a coelenterate glandular epithelium. *J. Gen. Physiol.*, **68**: 313–325.

MACKIE, G. O., AND D. A. BOAG, 1963. Fishing, feeding and digestion in siphonophores. *Pubbl. Stn. Zool. Napoli*, **33**: 178–196.

MACKIE, G. O., AND Q. BONE, 1976. Skin impulses and locomotion in an ascidian tadpole. *J. Mar. Biol. Assoc. U. K.*, **56**: 751–768.

MACKIE, G. O., AND G. V. MACKIE, 1967. Mesogleal ultrastructure and reversible opacity in a transparent siphonophore. *Vie Milieu*, **18**: 47–71.

MACKIE, G. O., AND L. M. PASSANO, 1968. Epithelial conduction in Hydromedusae. *J. Gen. Physiol.*, **52**: 600–621.

MORIN, J. G., 1974. Coelenterate bioluminescence. Pages 397–438 in L. Muscatine and H. Lenhoff, Eds., *Coelenterate biology. Reviews and new perspectives*. Academic Press, New York.

MORIN, J. G., AND J. A. COOKE, 1971a. Behavioural physiology of the colonial hydroid *Obelia*. II. Stimulus-initiated electrical activity and bioluminescence. *J. Exp. Biol.*, **54**: 707–721.

MORIN, J. G., AND I. A. COOKE, 1971b. Behavioural physiology of the colonial hydroid *Obelia*. III. Characteristics of the bioluminescent system. *J. Exp. Biol.*, **54**: 723–735.

MORIN, J. G., AND J. W. HASTINGS, 1971. Biochemistry of the bioluminescence of colonial hydroids and other coelenterates. *J. Cell. Physiol.*, **77**: 305–312.

MORIN, J. G., AND G. J. REYNOLDS, 1974. The cellular origin of bioluminescence in the colonial hydroid *Obelia*. *Biol. Bull.*, **147**: 397–410.

NICOL, J. A. C., 1958. Observations on luminescence in pelagic animals. *J. Mar. Biol. Assoc. U. K.*, **37**: 705–752.

PASSANO, L. M., 1973. Behavioral control systems in medusae; a comparison between Hydro and Scyphomedusae. *Publ. Seto Mar. Biol. Lab.*, **20**: 615–645.

PASSANO, L. M., G. O. MACKIE, AND M. PAVANS DE CECCATTY, 1967. Physiologie du comportement de l'hydroméduse *Sarsia tubulosa* Sars. Les sustèmes des activités spontanées. *C. R. Hebd. Séances Acad. Sci.*, **264**: 614–617.

PAVANS DE CECCATTY, M., J. M. BASSOT, A. BILBAUT, AND M. T. NICOLAS, 1977. Bioluminescence des élytres d'Acholoe. I: Morphologie des supports structuraux. *Biol. Cellulaire*, **28**: 57–64.

REYNOLDS, G. T., 1972. Image intensification applied to biological problems. *Q. Rev. Biophys.*, **5**: 295–347.

ROBERTS, A., AND C. A. STIRLING, 1971. The properties and propagation of a cardiac-like impulse in the skin of young tadpoles. *Z. Vgl. Physiol.*, **71**: 295–310.

ROMANES, G. J., 1876. Preliminary observations on the locomotor system of medusae. *Philos. Trans. R. Soc. Lond., B. Biol. Sci.* **166**: 269–313.

ROSE, B., AND W. R. LOEWENSTEIN, 1976. Permeability of a cell junction and the local

cytoplasmic free ionized calcium concentration: a study with aequorin. *J. Membr. Biol.*, **28**: 87–119.

SHERIDAN, J. D., 1973. Functional evaluation of low resistance junctions: influence of cell shape and size. *Am. Zool.*, **13**: 1119–1128.

SPENCER, A. N., 1974. Non-nervous conduction in invertebrates and embryos. *Am. Zool.*, **14**: 917–929.

SPURLOCK, B. O., AND M. J. CORMIER, 1975. A fine structure study of the anthocodium in *Renilla mülleri*. Evidence for the existence of a bioluminescent organelle, the luminelle. *J. Cell. Biol.*, **64**: 15–28.

STAEHELIN, L. A., 1974. Structure and function of intercellar junctions. *Int. Rev. Cytol.*, **39**: 191–283.